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14. ABSTRACT

The goal of this project is to deepen the understanding of the mechanisms and principles of formation of biotic-abiotic interfaces via controlled bio-assisted growth of nanostructured materials on templated, functionalized surfaces. Ultimately, we will utilize this advanced knowledge to design organized, integrated, and encapsulated nanostructured materials with complex 2D and 3D topologies for prospective biosensing applications in an interest of USAF.

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NANOSTRUCTURED INTERFACES FOR ORGANIZED MESOSCOPIC BIOTIC-ABIOTIC MATERIALS

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Project objectives

The goal of this project is to deepen the understanding of the mechanisms and principles of formation of biotic-abiotic interfaces via controlled bio-assisted growth of nanostructured materials on templated, functionalized surfaces. Ultimately, we will utilize this advanced knowledge to design organized, integrated, and encapsulated nanostructured materials with complex 2D and 3D topologies for prospective biosensing applications in an interest of USAF.

Specifically, our research plan is focused on the fundamental mechanism of surface-mediated formation of nanostructured materials facilitated by selective binding of biomolecules (peptides, proteins, and plasma polymerized polypeptides) gently tethered onto solid substrates via polyelectrolyte complexes with multiple weak interactions tunable by external stimuli. We will direct bio-assisted binding and growth within pre-designed polyelectrolyte matrices to obtain abiotic/biotic, nanostructured, inorganic or bi-metal nanoparticles, nanodots, and nanorods with desired structure, morphology, and properties by tuning confined space, interfacial conditions, and interactions. Finally, we will employ versatile fabrication routines to obtain 2D and 3D periodic and aperiodic biotic-abiotic mesostructures via microprinted capillary transfer and holographical lithographies combined with nanoscale encapsulation of nanostructured inorganic materials and probing their unique properties relevant to biosensing applications.

This cross-disciplinary project will enhance the collaboration with our partners from academic, national and AFRL, WPAFB. The collaboration with the AFRL groups such as sample exchange, complementary characterization, synthesis on new biomaterials, and interface/surface modification will be facilitated by mutual visits, student exchanges, joint student supervision, student summer internships at AFRL, joint presentations and publications, joint patent filing, and the mutual transfer of technologies relevant to long-term fundamental research interests at USAF.

Ongoing collaboration with three academic labs will bring biomolecules to the PI lab such as silk fibroin (D. Kaplan, Bioengineering Department, Tufts) and *rSilc* protein (N. Kroger, Chemistry and Biochemistry Department, GT), as well as unique spectroscopic characterization capabilities (M. El-Sayed, CBC, GT). The proximity of ORNL and initiated contacts will facilitate the availability of this neutron source with the highest flux for unique measurements.

Students participating in the project will constantly interact with researches at AFRL by numerous means including joint supervision and GT-AFRL summer internships. They will mature as multifaceted researchers deeply involved into USAF-relevant fundamental studies in highly multidisciplinary fields of biotechnology, materials science, nanotechnology, and biochemistry. The synergism of joint collaborative efforts of the PI group and highly skilled AFRL research groups will offer invaluable experience to graduate students and enhance a prospective workforce training for the USAF.

DoD potential enhancement: The gained knowledge will be a fundamental basis for prospective ultra-sensing platform from hybrid organized nanomaterials for chemical, optical, and biological applications with potential for dramatic miniaturization and superior sensitivity of lightweight hybrid sensor arrays.   Students trained in this field will form a pool of workforce for AFRLs.

Major focus areas
Specifically, our research plans include several specific targets on:
• understanding fundamental mechanisms of the surface-mediated formation of nanostructured materials induced by selective binding of biomolecules (peptides, proteins, and plasma polymerized polypeptides) tethered to functionalized polyelectrolyte templates.
• directing the nanostructure nucleation and growth with tunable, layer-by-layer (LbL)-mediated templates screened from solution-based growth is a signature mark of this research. The fundamental knowledge of surface-nanoparticle interactions is utilized to tailor bio-assisted surface processes within pre-designed polymer matrices to obtain hybrid nanostructured materials with desired structure, morphology, and properties.
• understanding of hierarchical nanostructural organization, composition, and multi-length scale morphologies of several selected inorganic materials including bi-metal nanoparticles (Au-Ag, Au-Pd) as well as TiO_2, ZnO nanodots and nanorods, which are of interest of prospective applications in photosensing, photocatalytic, and biosensing fields.
• applying versatile fabrication routines to obtain 2D and 3D periodic and biotic-abiotic mesostructures with complex shape and controlled architectures by employing microstamping, microprinted capillary transfer lithography, and holographical lithography as well as an encapsulation of nanostructured inorganic materials within flexible meso-structures and the creation of continuous organized networks integrating biomolecules, inorganic nanostructured materials, and polymer matrices.
• probing the physical and chemical properties of these biotic-abiotic structures (optical response, spectral signature, selective binding) relevant to sensing applications and sprouting from the intricate organization, high surface to volume ratio, and unique combination of biological molecules and inorganic nanophases.

Major accomplishments
• For synthetic polyelectrolyte templates, we found that layer stratification and the degree of layer interpenetration can be controlled by varying type and concentration of salt during LbL assembly. Our results uncovered predominant stratification of molecular layers in spin-assisted LbL films. We also found spin-assisted LbL films obtained from buffer solutions to be more stratified as compared to interpenetrated layers in the conventional LbL films.
• We prepared redox-active nanoscale LbL films with polyaminoacid-decorated surfaces which serve for both nucleation and growth of uniformly distributed gold nanoparticles at ambient conditions. We found that a poly-L-tyrosine (pTyr), a synthetic polyaminoacid, was able to direct nanoparticle formation to solid, flexible, and patterned surfaces preventing

particle agglomeration. The gold particles were of 8±2 nm in diameter, surrounded by 3-6 nm polyaminoacid shell.

• We demonstrated that a recombinant protein, silaffin rSilC, adsorbed on a tailored polyelectrolyte surface was capable of initiating the nucleation and growth of 4-nm titania nanoparticles in a uniform and dispersed manner under ambient conditions. The growth of titania nanoparticles on the surface appeared to be confined within the rSilC assemblies thus preventing the uncontrolled formation of large nanoparticle aggregates.

• In related study we utilized silk as an ultrathin redox-active template for controllable, one-step synthesis of gold nanoparticles via control over silk secondary structure. We found that both silk I and silk II molecular layers can facilitate gold nanoparticle formation at ambient conditions, indicating that tyrosine groups are available for metal ion reduction in both forms of silk.

• We have demonstrated that the secondary structure of silk films can be patterned on the micron scale using a soft lithographic technique with high spatial resolution. The selective transformation of the silk I structure resulted in well-defined regions with very differed mechanical, surface, and solubility properties controlled by local secondary structure of silk protein.

• We introduced a novel method for creating conformal, ultrathin, uniform aminoacid (tyrosine) films on a variety of practical surfaces via plasma enhanced chemical vapor deposition. During the plasma deposition, tyrosine is sublimed into the plasma field and directly deposited uniformly on different substrates forming a conformal coating in a one-step, solvent-free process. This allows many practical surfaces to be modified so that they are capable of initiating gold nanoparticle formation while avoiding a wet chemistry treatment. Micropatterning of the tyrosine PECVD coating achieved by placing a mask is exploited for the creation of corresponding patterned gold nanoparticles.

• Crosslinked silk films and monolayers from silk block-copolymers have been fabricated and their enhanced mechanical properties and organized supramolecular structures have been studied.

• ZnO nanostructures of different types have been synthesized by wet chemistry approach as assisted by synthetic polyelectrolytes and zinc-binding peptides and compared with conventional vapor-phase method. Core-shell structures and dense arrays have been grown and Raman mapping was developed for in-situ probing of facet orientations.

• Finally, we have demonstrated probe-free and highly sensitive nanowire-based sensors for detecting biologically- and chemically-charged molecules based upon the non-symmetrical Schottky contact under reverse bias. Such a design does not need a bio-probe to detect molecules; rather, it depends on the attraction of the charged molecules to the junction region and it has the potential of proactively detecting charged molecules with a low detection limit of 2 fg/ml.

Selected detailed examples of studies conducted

Organized LbL templates: inner microstructure. Despite these systems have been extensively employed in the past years, the fundamental understanding of the mechanism of film formation is still in their infancy. It is generally suggested that strong shear forces combined with fast solvent removal are responsible for enhanced mechanical integrity of these films but, however, no direct evidences on unusual layered organization in these films have been published to date. Charr et al, in his pioneering work examined internal structure of spin-assisted LbL with inorganic nanoparticles incorporated within the layers at different distances. The results illustrated that that layers of inorganic nanoparticles were resolved
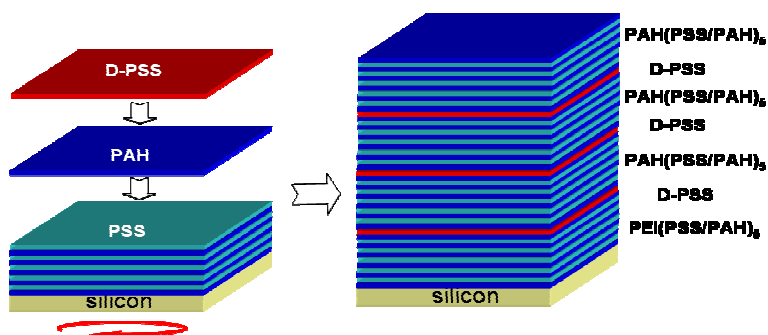
Fig.1. Graphic representation of spin-assisted layer-by-layer assembly and resulting multilayer architecture with selectively deuterated PSS layers.

between the polyelectrolyte layers. However, the data obtained for hybrid organic-inorganic systems can not present a direct evidence of well-resolved polyelectrolyte layers in the spin-assisted LbL films. For the accurate analysis of internal structure within all-polymer spin-assisted LbL assemblies, neutron reflectivity has to be exploited.

We demonstrated that the degree of intermixing within spin-assisted LbL films can be tuned by type and concentration of salt in the deposition solutions. We found highly stratified structure with well-pronounced layering in spin-assisted LbL films obtained from salt-free solution (Fig. 2). However, the internal structure can be drastically changed from stratified to interdiffused in the presence of a small amount of phosphate ions (10 mM), and, in turn, changed back to the highly stratified structure by adding NaCl to the buffer solution during deposition. Moreover, the presence of phosphate buffer affects the structure by increasing degree of interdiffusion. At the same time, the spin-assisted LbL films deposited from buffer show more persistent layering than that in conventional LbL films. We also found that, introducing of NaCl into the phosphate buffer increases the internal and external roughness of the spin-assisted LbL films as compared to those for the salt-free films. At the same time the presence of NaCl in phosphate buffer during LbL film construction allows obtaining thicker well-stratified films.
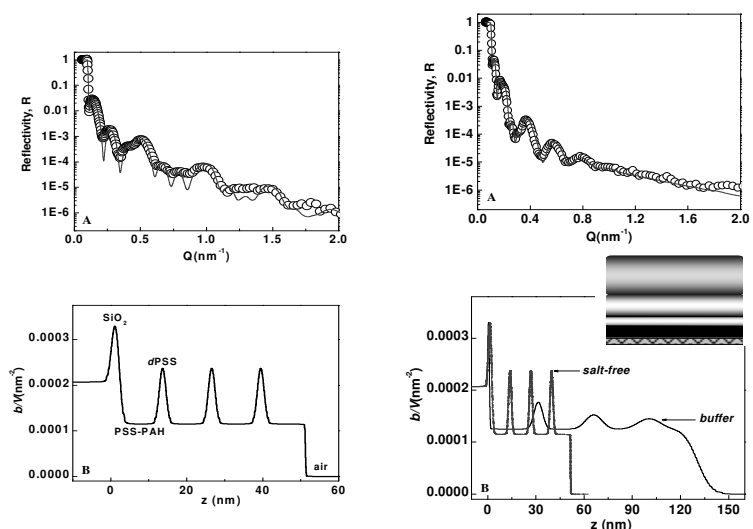

Fig. 2. Examples of neutron reflectivity data for SA-LbL films with embedded deuterated PPS marker obtained from salt-free solution

Bio-assisted and surface-mediated growth of inorganic nanoparticles. The use of biomolecules as templates for the synthesis of inorganic nanoparticles has the potential to enable precise control over particle size, aggregation state, and spatial arrangement. Syntheses of TiO_2 (titania) nanoparticles are of particular interest, because of its numerous applications as photocatalyst, UV blocker, oxygen sensor, as well as material for lithium batteries and solar cells. Previously, the proteins silicatein, lysozyme, silaffins, and amino-acids have been employed for titania synthesis at ambient temperature. For example,

Kröger et al. have recently shown that the recombinant silaffin protein, rSilC, enabled the synthesis of aggregates of titania nano- and microparticles at ambient temperature and neutral pH in aqueous solutions. However, the mechanism of biomolecule-mediated titania synthesis especially in the presence of nanostructured surfaces is not yet understood.

In current study, we have shown that rSilC protein deposited on a properly charged surface of polyelectrolyte LbL films initiates nucleation and growth of titania nanoparticles in a uniform and dispersed manner at ambient conditions (see Scheme 1). We suggest that the confinement of titania nanoparticles into nanoscale protein surface domains limits nanoparticle growth preventing formation of larger microscopic aggregates.

Indeed, the 6 nm nanoparticles obtained by induction through surface-bound proteins (Fig. 3) are composed of 4 nm titania cores surrounded by 1 nm protein shells. We suggest that the approach presented here can be readily expanded to other organic and inorganic materials to enable a simple and robust method of synthesis of uniform, surface organic-inorganic nanostructures with controllable dimensions and surface distribution.

Fig. 3. AFM and TEM images of silk domains and titania nanoparticles formed and corresponding schematic.

Another study conducted here showed that PECVD provides a facile method for the deposition of tyrosine for reducing gold nanoparticles on a surface. Smooth, uniform films have been shown to be able to be created quickly and reliably through this method. Additionally, PECVD is a one step coating process that completely eliminates the need for any wet chemistry in fabrication of the tyrosine film since the solid monomer can be heated and sublimed directly into the plasma stream during deposition. This method forms highly crosslinked polytyrosine during the deposition as shown in the chemical analysis which contributes significantly to the films robustness.

In fact, the plasma deposited tyrosine films have shown excellent robustness when exposed to gold chloride solution for mineralization, demonstrating the versatility and durability of this surface coating technique for nanoparticle reduction on a variety of substrates from silicon to PTFE. Pattering of tyrosine films demonstrates that the PECVD/mask system combination is an effective method to create patterned regions on the substrate that are capable of
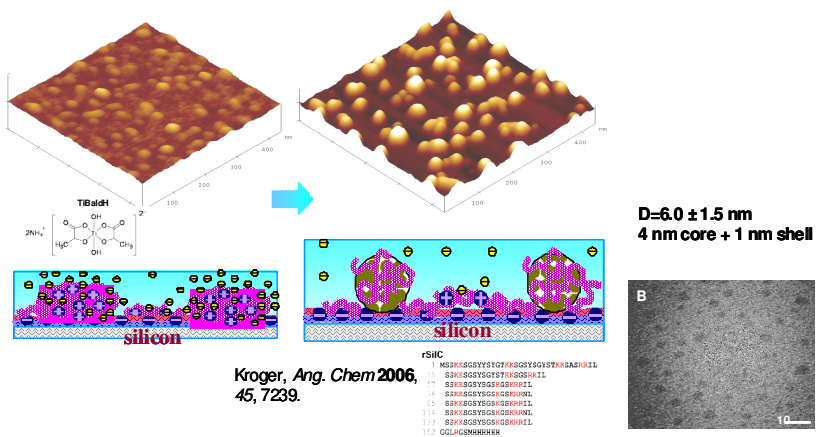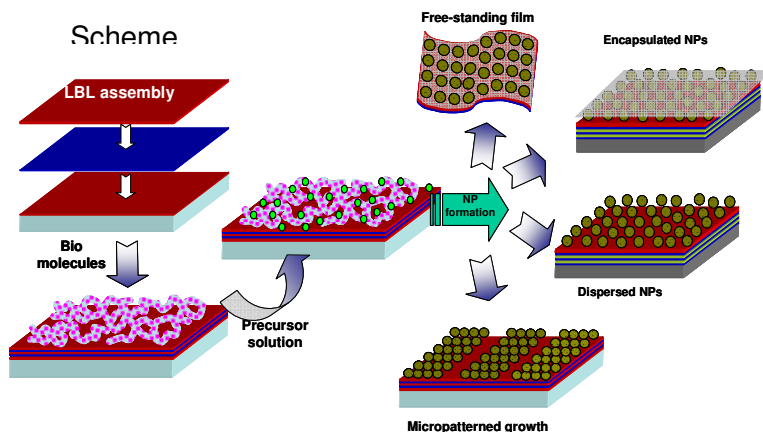
selectively reducing gold nanoparticles on the surface (Fig. 4).   This demonstrates the potential for precisely directing the areas of growth of gold nanoparticles that is controlled simply by the pattern laid down on the surface. While only square patterns were created and studied in this study, there is a large range of potential shapes and patterns that can be created and this method can be extended to complex shapes such as ones used for micro and nanolithography patterning.
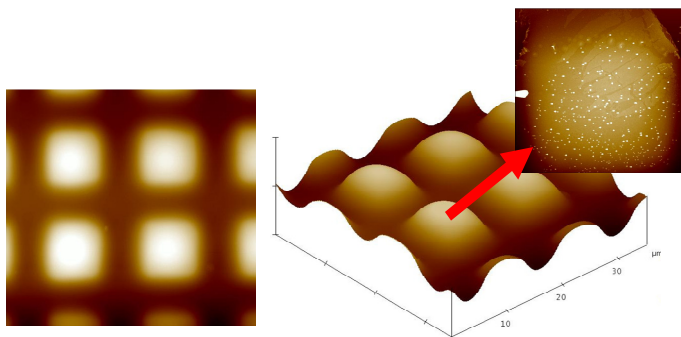

Fig. 4. Micropatterned polytyrosine PECVD coating and an example of gold nanoparticle grown on a selected square area.

Plasma polymerized polyaminoacid coatings have a great potential for tunable organic/inorganic composite material fabrication since the inorganic reducing amino acid monomer can be coated onto any substrate to which inorganic particle are desired to be attached. This method of selective gold nanoparticle formation has been also adaptable to a variety of materials such as silicon, glass, cellulose, PS, PDMS, and Teflon which otherwise cannot be conformally coated with uniform aminoacid and utilized for further biometallization.

Silk-based nanostructured materials.   Silk-based materials with incorporated metal nanoparticles are of special interest since the nanocomposites have been used for a range of biomedical and sensing applications, such as anti-bacterial coatings, thin film sensors, and plastic "memory" devices. Silk templates were utilized as a matrix for incorporation of pre-formed silver nanoparticles or for binding metal ions from solutions and their subsequent chemical reduction. Silk was also found to reduce metal ions from aqueous solution without the need of additional reducing agents, resulting in one-step *in situ* synthesis of metal nanoparticles at ambient conditions. This technique was applied to silk fibroin solutions at alkaline conditions to obtain colloidal core-shell gold-silk nanoparticles of high monodispersity. Silk microfibers with diameters of 2-5 μm have also been used as solid templates for reduction of silver and gold nano- and microparticles.

Despite major advancements in bioenabled synthesis of nanostructured materials, the synthesis of monodisperse, non-aggregated nanoparticles at the surface of solid substrates at ambient conditions remains a significant challenge.   The prior work on silk-templated mineralization has mostly focused on μm-sized silk films or fibers. In addition, the size of inorganic particles on silk surfaces usually exceeded several microns with a broad particle size distribution and significant aggregation. Furthermore, the role of silk secondary structure on metal reduction as well as possible alternation of silk conformation as a result of
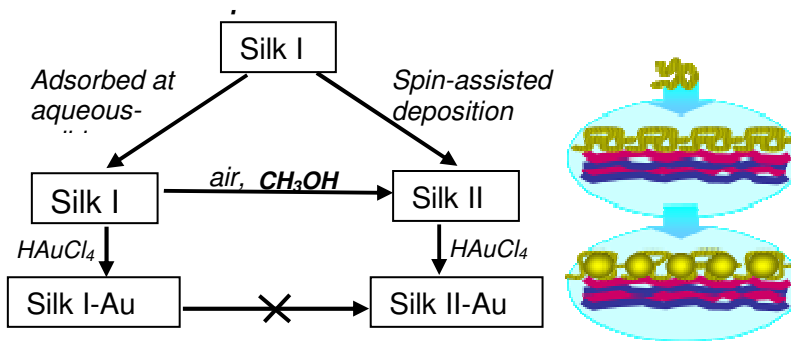

Fig. 5. Transitions from silk I into silk II induced by deposition conditions, pos-depositional treatments, or gold reduction.

metal reduction were not investigated (Fig. 5).

Therefore, in our study, we focus on silk nanostructures under different conditions (Fig. 5) and found that both silk I and silk II molecular layers can facilitate gold nanoparticle formation at ambient conditions, indicating that tyrosine groups are available for metal ion reduction in both forms of silk.

Silk materials (silk I and amorphous silk) can undergo an irreversible transition in secondary structure of silk II type upon exposure to organic solvents, heat, dehydration, and mechanical strain. Silk II as a partially crystalline material with nanometer-scale crystallites which shows increased elastic modulus and mechanical strength which all are critical for demanding mechanical applications. We suggest that the presence of ß-

Fig. 6. ATR-FTIR spectra of a silk molecular layer obtained in D_2O and further exposed to air.

sheets in silk II facilitate tyrosine ordering thereby resulting in well-dispersed, uniform nanoparticles with diameters less than 6 nm. In addition, the mineralization does not result in transformation of the silk I secondary structure to silk II. In fact, the silk I structure is stabilized from further transformation into silk II even upon drying as was monitored by in-situ ATR-FTIR (Fig. 6). These results are critical for developing a better understanding of silk interfacial behavior and offer an opportunity to design a new class of nanocomposites that combine the beneficial features of silk with those of the nanoparticles.

The rational design of nanocomposite materials and tailored surfaces which combine properties of these two different silk phases in an organized manner is an intriguing opportunity which has not been explored to date. Thus we developed a novel strategy to selectively tune the chemical and mechanical properties of nanoscale silk fibroin materials by spatial micropatterning of the protein's secondary structure with high resolution. Furthermore, we show that the patterned silk material with alternating silk I and silk II regions retain their intrinsic chemical and mechanical characteristics and show well-developed modulation of localized properties. With this strategy, we envision the ability to fabricate structured silk materials with properties tuned in the range between silk I and silk II as well as micropatterned silk materials with highly localized regions tailored to suit particular functions. The ability to create tailored biocompatible materials with tunable properties in a straightforward and robust way without the use of toxic chemicals will provide additional versatility to the use of silk in biotechnology and microdevices applications.

The technique for patterning silk secondary structure presented here utilizes capillary transfer lithography (CTL) as a first step (Figure 7). By utilizing CTL we first deposited a sacrificial polystyrene (PS) mask onto a flat silk I film which was spin-cast from aqueous solution on silicon wafer. The masked silk I film was then briefly exposed to methanol vapor,
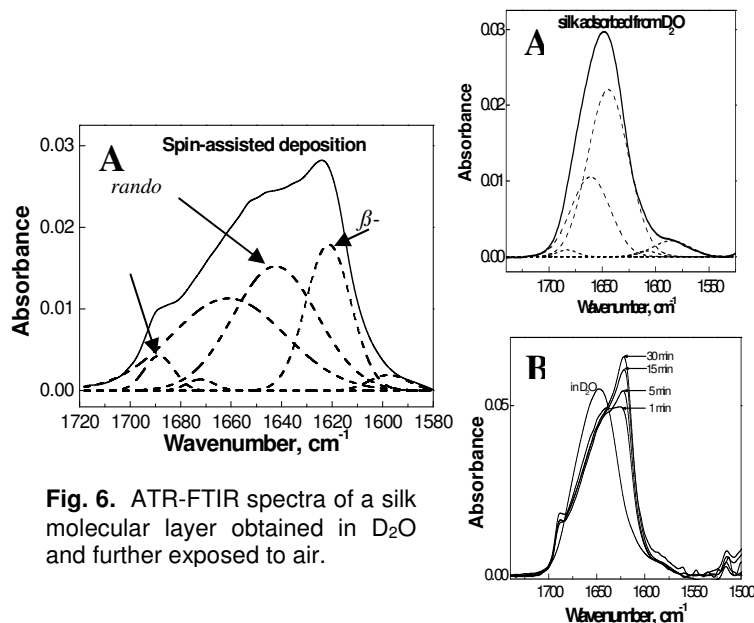
which induced a highly localized transformation to silk II in the exposed regions without affecting other regions protected by PS mask. The PS mask was then dissolved away leaving a flat silk film with coexistent silk I and silk II regions alternating in a manner determined by the pattern of the original mask (Figure 7).
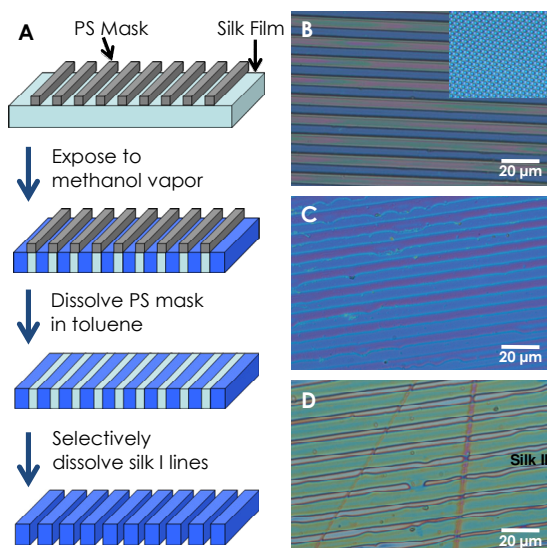

Fig. 7. Strategy for patterning secondary structure in silk films. a) Schematic of the patterning strategy, from top to bottom. First, a PS mask is deposited on the silk film by CTL. Next, the silk film is selectively exposed to methanol vapor converting the exposed regions to silk II. Third, the PS mask is removed in toluene. Finally, the silk I regions can be selectively dissolved in water to reveal the patterning. b) Optical image of silk film with PS mask deposited on top (inset is the square pattern at the same scale). c) Optical image after removal of the PS mask in toluene. d) Optical image after selective dissolution of the silk I regions.

We have demonstrated that this way the secondary structure of silk films can be patterned on the micron scale with high spatial resolution. The selective transformation of the silk I structure resulted in well-defined regions with differed mechanical, surface, and solubility properties controlled by local secondary structure of silk protein. We believe that while the silk substrates used in this study were flat films, this method can be extended to other geometries. A variety of other microprinting techniques including inkjet printing, dip-pin lithography, nanosphere lithography, and photo lithography could be used to expand the applicability of this approach to other surfaces with higher resolution and over larger areas. In the future, this technique will allow researchers to study the properties of silk I and silk II side by side and allow for the development of tailored protein materials with nominally uniform chemical composition but selectively transformed secondary structure.

ZnO-nased nanostructures. We developed a novel method for direct, fast, non-ambiguous, and nondestructive identification of the growth direction and orientation of *individual* ZnO nanostructures in the device-ready environment by exploiting high resolution confocal Raman mapping. Various features of the Raman spectrum of ZnO nanostructures, vapor deposition grown nanobelts and peptide-assisted vertical nanorods, were found to be sensitive to the relative orientation of the crystal plane. Furthermore, we discovered that the waveguiding property of the ZnO nanobelt is also orientation dependent and results in either enhancement or suppression of Raman scattering from the underlying substrate. We demonstrate that various features of Raman spectrum of ZnO and the modulation of the substrate signal can be employed for the rapid and non-destructive identification of the crystal growth direction and orientation of these nanostructures even after integration into devices, which is impossible with current electron microscopy and diffraction techniques.

We fabricated a miniature, electrical readout, and highly sensitive temperature sensor/switch, based on ZnO nanobelt/plasma-polymerized benzonitrile bimorph structure suspended between two electrods. A new readout technique based on the change in the electric current flowing through the bimorph and the contact pad has been employed replacing conventional cumbersome peizoresistve method. The thermal sensor constructed

is highly prospective for thermal switching and triggered detection owing to the relative ease in the fabrication of microscopic arrays.

In another study, we deliberately introduced a non-symmetrical Schottky contact at one end of a ZnO nanowire nanodevice and studied its response to biomolecules. The results show its much-enhanced sensitivity in comparison to ZnO nanowires with Ohmic contacts. The operation of the nanosensor is largely determined by the property at the Schottky junction and it can clearly differentiate between positively- and negatively-charged molecules; this is a major difference from the nanosensors' characteristics as reported in the literature. The electrical response comes from the variation of the Schottky barrier height (SBH) and barrier width as a result of biomolecule adsorption at the Schottky contact. Such a nanosensor is probe-free, and there is no need to introduce antibody-antigen interaction for enhancing its sensitivity. This introduces a new methodology in the design of nanosensors.

Our nanosensors were fabricated using ZnO NWs grown by a vapor-solid process. For comparison purposes, we have fabricated an Ohmic contact device (OCD) and Schottky-gated device (SGD) using the same type of NWs. For OCD, a single crystal ZnO NW was placed on a Pt electrode pattern, and then Pt-Ga was deposited on the both sides of the NW to form Ohmic contacts using a focus ion beam (FIB) system. The length of the NW was sufficiently long to minimize the effect from FIB contamination. The mechanism of OCD is to detect molecules by measuring the variation of the conductance. We used hemoglobin as our sensing molecules; the pH was adjusted to control the electrical properties of the molecules. When the positively- or the negatively-charged molecules was introduced at a concentration of 800 µg/ml, the electrical signals of the device showed little change. This was possibly because the conductance of the ZnO NW was rather high, so that an introduction of surface adsorbed molecules did not change the conductance to any appreciable level.

It is apparent that, as for the ZnO nanowire-based sensors, the response of the SGD is much larger than that of the OCD. The results of SGD were reproduced multiple times. Figure 8 shows the repeated detection of the positively-charged molecules at a fixed concentration of c = 800 µg/ml under an applied reverse bias of 0.5 V. The positively-charged molecules increase the conductance in reference to DI water. The repeatedly-congruent results show the stability of the sensor. A response factor of around 800% has been received.

Fig. 8. A repeated detection of the positively-charged molecules at a concentration of c = 800 □g/ml under an applied reverse bias of 0.5 V.

The response of the SGD to charged molecules can be explained from its band diagram in the area of metal-nanowire contact. Once the negatively-charged molecules are being adsorbed at the junction region, the local Schottky barrier height and width are increased (by Δ') due to the local build up of negative molecules. In such a case, the conductance of the device drops as more negatively-charged molecules are adsorbed at the junction region, and thus the magnitude of the transported current drops in reference to the DI water case. On the other
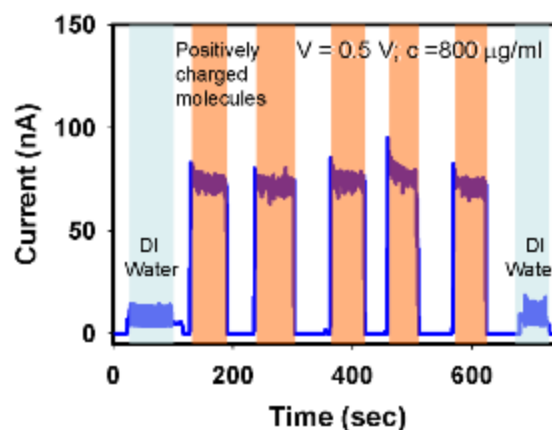
hand, when the positively-charged molecules are introduced in the solution, the positively-charged molecules lower the SBH (by Δ) at the contact region although that may increase the barrier width, but it is the height of the barrier that matters to the charge transport at room temperature. The conductance of the junction increases dramatically, resulting in an increase in the transported current as shown experimentally in Fig. 8.

Converting Biomechanical Energy into Electricity by Muscle Driven Nanogenerator: A living species has numerous sources of mechanical energy, such as muscle stretching, arm/leg swings, walking/running, heart beats and blood flow. We demonstrate a piezoelectric nanowire based nanogenerator that converts biomechanical energy, such as the movement of a human finger and the body motion of a live hamster (Campbell's dwarf), into electricity. A single wire generator (SWG) consists of a flexible substrate with a ZnO nanowire affixed laterally at its two ends on the substrate surface. Muscle stretching results in the back and forth stretching of the substrate and the nanowire. The piezoelectric potential created inside the wire leads to the flow of electrons in the external circuit. The output voltage has been increased by integrating multiple SWGs. A series connection of four SWGs produced an output voltage of up to ~0.1- 0.15 V. The success of energy harvesting from a tapping finger and a running hamster reveals the potential of using the nanogenerators for scavenging low-frequency energy from regular and irregular biomotion.

Large-scale lateral-nanowire-arrays integrated flexible nanogenerators: An innovative approach that combines a rational chemical growth of nanowires (NWs) with nanofabrication for building multiple lateral-nanowire-arrays integrated nanogenerators (NGs) for large-scale and high output energy harvesting. The entire fabrication uses a flexible and stretchable polymer as substrate and the processing temperatures at the substrate is lower than 100 $^{\circ}$C. A maximum voltage output of 1.26 V has been demonstrated, which is a landmark progress towards self-powered nanosystems. ZnO nanowires (NWs) are unique candidates not only for fabricating nanosensors, but also for scavenging mechanical energy. We demonstrate an integration of NW based nanogenerator (NG) and NW nanosensor (s), aiming at building up solely NW-based, self-powered nanosystems. An alternative current (AC) NG is fabricated using vertically aligned ZnO NW arrays that are fully contacted at the two ends and well packaged by soft materials. The presence of a Schottky contact at one electrode at least leads to periodic charge accumulation and releasing as the NWs being stressed and released by a dynamic deformation. Integrating three layers of such AC-NGs creates an open circuit output voltage of 0.243 V and a peak power density of 2.7 mW/cm^3 under a low-frequency agitation.

Mirror and transparent nanocomposites. We report on a novel type of *all-natural bio-compatible and very robust* nanoscale free-standing biohybrids from alternated layered silk monolayers and clay nanoparticles. These reinforced silk-clay films were obtained by integrating a silk fibroin matrix with functional inorganic nanoplatelets using spin-assisted layer-by-layer assembly (Figure). Organized assembly of silk protein with clay (montmorillonite) nanosheets resulted in the highly transparent nanoscale films with significantly enhanced mechanical properties, including strength, toughness and elastic modulus, all many-fold higher than that for pristine silk nanomaterials. Moreover, replacing clay nanoplatelets with a highly reflective Langmuir
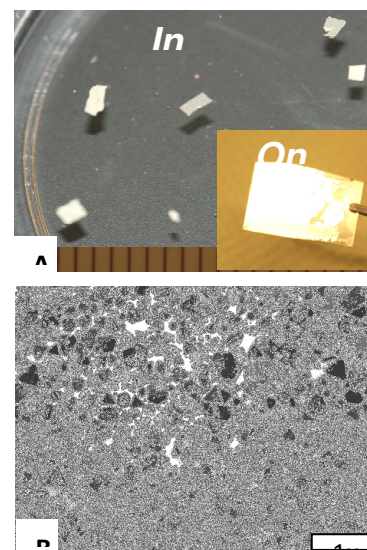

Optical (A) and TEM (B) images of free-standing silk$_{10}$-AgNPL-silk$_{10}$ films.

monolayer of densely packed silver nanoplates caused similar enhancement of mechanical properties but, in contrast, created a highly-reflective, mirror-like nanoscale flexible films, a unique property of silk materials with thicknesses around 100 nm. This strategy offers new perspective for the fabrication of robust all-natural biocompatible flexible nanocomposites with exceptional mechanical properties important for biomedical applications such as reinforced tissue engineering. On the other hand, the ability to convert silk-based nanoscale films into mirror-like biocompatible flexible films can be intriguing for prospective photonics and optical exploitation of these nanobiohybrids.

Co-cross-linked silk nanocomposites. A novel assembly approach to fabricate ultrathin, robust, freely-standing nanocomposites was demonstrated. Pre-cross-linked silk fibroin ultrthin films incorporated with silica nanoparticles with silsesquioxanes cores (POSS) or clay nanoplatelets were fabricated via a one-step, one-solution layer-by-layer (LbL) approach. The reinforced silk membranes have enhanced mechanical properties compared to traditional silk-based nanocomposites previously reported. While traditional LbL-assembled silk-based nanocomposites showed only a 3-fold increase in mechanical strength, the nanocomposites reported on

here demonstrated up to 6-fold and 8-fold increases in elastic modulus and toughness, respectively. These silk-based nanocomposites also exhibited excellent optical transparency in the visible region, especially when reinforced with POSS nanoparticles, which suggests potential usefulness as low cost, non-toxic, and easily scalable reinforced biotmaterials for mechanically demanding applications.
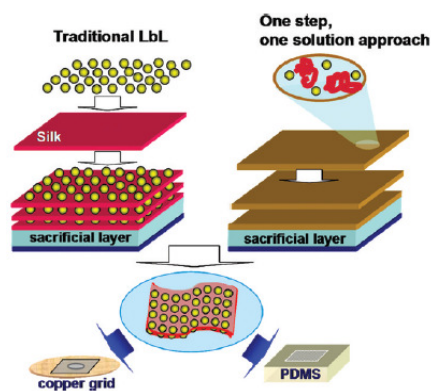
Graphene oxide-polyelectrolyte nanomembranes. We demonstrate that the mechanical properties of ultrathin, laminated nanocomposites can be significantly enhanced by the incorporation of small amounts of a dense monolayer of graphene oxide flakes (GO). Graphene oxide is considered to be an excellent two-dimensional filler for polymer nanocomposites because of its remarkable mechanical properties. Negatively-charged graphene oxide layers were incorporated into polyelectrolyte multilayers (PEMs) fabricated in a LbL assemby via Langmuir-Blodgett deposition. These LbL-LB PEM-GO nanocomposite films were capabable of being released as robust, freely standing membranes with large lateral dimensions

on the order of centimeters and thicknesses on the order of 50 nm. A critical limitation of traditional nanocomposite fillers is that the loading fraction required for optimum mechanical enhancement is high, and can often reach up to 50%. These nanocomposite films, however, showed optimum increases in toughness from 400 kJ·m^{-3} for a PEM thin film with no GO content, to 1800 kJ·m^{-3} for a PEM thin film loaded with 3.3 vol% GO flakes. Marked increases in elastic modulus were also observed ranging from 1.5 GPa for pure PEM membranes to about 20 GPa for only 8 vol% loading content of GO. The tough nanocomposites show great potential for mechanical applications with require high performane thin film nanocomposites.
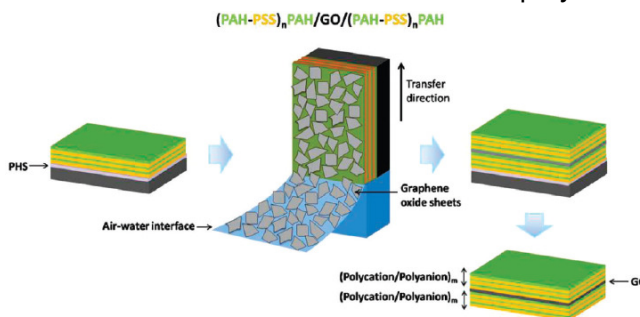
LbL hydrogels with confined quantum dots. We report on responsive photoluminescent hybrid materials with quantum dots immobilized in an prganized manner by spin-assisted layer-by-layer assembly. The strongly interacting polyelectrolytes, poly(allylamine hydrochloride) (PAH) and poly(sodium 4-styrenesulfonate) (PSS), serve for confining CdTe nanoparticles stabilized by thioglycolic acid, while a poly(methacrylic acid) (PMAA) hydrogel matrix presents an elastomeric network with pH-responisve properties. Quantum dot layers encapsulated in PSS-PAH bilayers are confined inside this hybrid hydrogel network. The system undergoes reversible changes in photoluminescent intensity in response to pH variations. The network's photoluminescent intensity is suppressed in excess negative charge in high pH, while excess positive charge in low pH conditions results in an significant increases in photoluminescence.
Such hybrid quantum-dot containing hydrogel-LbL assemblies provide a way for a novel design of materials with precisely controlled structure and pH-triggered optical properties which could be used as pH or chemical sensors.
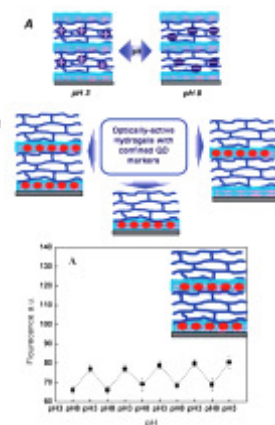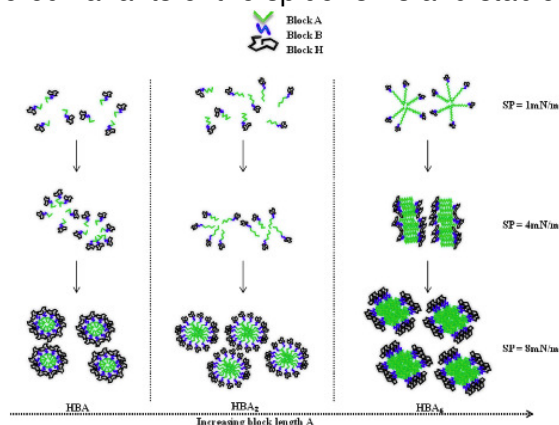
Periodic porous polymer-nanoparticle structures. We have demonstrated that periodic, porous polymer microstructures composed of 3D polymer templates can be decorated with metal nanoparticles by their in-situ growth on aminoacid or inside synthetic phase. The direct growth on both the outer and inner surfaces of these structures is enabled by the biofunctionalization with uniform polyaminoacid coatings (Figure). By applying either liquid- or vapor based biofunctionalization of highly porous polymer structures, we achieved the nanoparticle coverage reaching 220 NP/μm^2 The diameter of gold nanoparticles as well as surface coverage can be controlled by the variation of parameters of aminoacid coatings. The preliminary mechanical measurements reported here clearly reveal the stiffening of the surface. In another study, in-situ growth on gold nanoparticles was conducted inside of individual nanotubes composed of synthetic PS-PVP block copolymers which show pH-sensitive behavior.
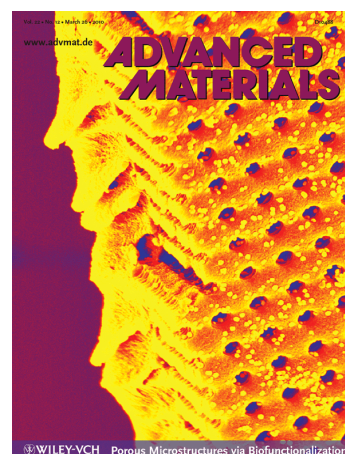
Decorating gold nanoparticles on aminoacid-modified periodic porous polymer.

Thin film assembly of spider silk-like block copolymers
We report the self-assembly of monolayers of spider silk-like block copolymers. Langmuir isotherms were obtained for a series of bioengineered variants of the spider silks and stable monolayers were generated. Langmuir Blodgett films were prepared by transferring the monolayers onto silica substrates and were subsequently analyzed by atomic force microscopy (AFM). Static contact angle measurements were performed to characterize interactions across the interface (thin film, water, air) and molecular modeling was used to predict 3D conformation of spider silk block copolymers. The influence of molecular architecture and volume fraction of the protein spider silk block copolymers on the self-

assembly process were assessed. At high surface pressure, spider silk block copolymers with minimal hydrophobic block (fA = 12%) formed oblate structures, whereas block copolymer with a six-fold larger hydrophobic domain (fA = 46%) formed prolate structures. The varied morphologies obtained with increased hydrophobicity offer new options for biomaterials for coatings and related options. The design and use of bioengineered protein block copolymers assembled at air-water interfaces provides a promising approach to compare 2D microstructures and molecular architectures of these amphiphiles, leading to more rationale designs for a range of nanoengineered biomaterial needs, as well as providing a basis of comparison to more traditional synthetic block copolymer systems.

Personnel training and collaboration with AFRL researchers
MSE graduate students, who participated in the project and graduated in 2009-2010 are: Dr. M. McConney (PhD, 2009, an NRC post-doc fellow at AFRL), Dr. S. SIngamaneni (PhD, 2009, faculty member at WashU, St. Louis), and Dr. E. Kharlampieva (2010, faculty member at U. Alabama). All current project participants, M. Gupta, K. Anderson, S. Young, and B. Wallet are involved in close collaboration with AFRL researchers.

Current AFOSR project promotes intense, inspiring, and mutually benefitial collaboration of the PI's group and AFRL researchers. In fact, one student (McConney) who graduated from the PI's group in 2009 moved as an NRC post-doctoral Fellow to AFRL (Bunning), one researcher from AFRL (Gupta) became a PhD student in the PI's group. Two students (Anderson and Gupta) are co-advised by AFRL researchers who also serve as adjunct professors at GT (Bunning and Naik). Three students conducted summer research at AFRL to enhance collaboration and were supported by AFOSR supplement. In addition, two AFRL researchers visited the PI's lab to conduct experiments and discuss results. In the course of the project, the students were involved in fruitful collaboration with Kaplan's and Kroger's groups and with AFRL researchers (Naik's and Bunning's groups).

Peer-reviewed project publications:
1. S.T. Krishnaji, W. Huang, O. Rabotyagova, E. Kharlampieva, I. Choi, V.V. Tsukruk, R. Naik, P. Cebe, D.L. Kaplan, Thin Film Assembly of Spider Silk-like Block Copolymers, *Langmuir*, **2011**, *27*, 1000–1008.
2. E. Kharlampieva, V. Kozlovskaya, B. Wallet, V.V. Shevchenko, R.R. Naik, R. Vaia, D.L. Kaplan, V.V. Tsukruk, Co-cross-linking Silk Matrices with Silica Nanostructures for Robust Ultrathin Nanocomposites, *ACS Nano*, **2010**, *4*, 7053**–**7063.
3. E. Kharlampieva, V. Kozlovskaya, R. Gunawidjaja, V.V. Shevchenko, R. Vaia, R.R. Naik, D.L. Kaplan, V.V. Tsukruk, Flexible Silk-Inorganic Nanocomposites: From Transparent to Highly Reflective*, Adv. Funct. Mater.*, **2010**, *20*, 840–846.
4. D.D. Kulkarni, I. Choi, S.S. Singamaneni, V.V. Tsukruk, Graphene Oxide-Polyelectrolyte Nanomembranes, *ACS Nano*, **2010**, *4*, 4667–4676.
5. E. Kharlampieva, V. Kozlovskaya, O. Zavgorodnya, G.D. Lilly, N.A. Kotov, V.V. Tsukruk, pH-responsive photoluminescent LbL hydrogels with confined quantum dots, *Soft Matter*, **2010**, *6*, 800–807.
6. M. K. Gupta, S. Singamaneni, M. McConney, L. F. Drummy, R. R. Naik, V. V. Tsukruk, Silk Materials with Patterned Secondary Structure, *Adv. Mater.*, **2010,** *22,* 115-119.
7. S. Singamaneni, E. Kharlampieva, J.-H. Jang, M. E. McConney, H. Jiang, T. J. Bunning, E. L. Thomas, and V. V. Tsukruk, Metallized Porous Interference Lithographic Microstructures via Biofunctionalization, *Adv. Mater.*, **2010,** *22*, 1369-1373.
8. E. Kharlampieva, V. Kozlovskaya, R. Gunawidjaja, V. V. Shevchenko, R. Vaia, R. R. Naik, D. L. Kaplan, V. V. Tsukruk Flexible Silk-Inorganic Nanocomposites With Transparent to Mirror-like Optical Properties, *Adv. Funct. Mater.,* **2010,** *20,* 840-846.

9. S. Chang, S. Singamaneni, E. Kharlampieva, S. L. Young, V. V. Tsukruk, Responsive Hybrid Nanotubes Composed of Block Copolymer and Gold Nanoparticles, *Macromolecules*, **2009**, *42*, 5781

10. K. D. Anderson, J. M. Slocik, M. E. McConney, J. O. Enlow, R. Jakubiak, T. J. Bunning, R. R. Naik, V. V. Tsukruk, Facile Plasma Enhanced Deposition of Ultrathin Crosslinked Amino Acid Films for Conformal Biometallization, *Small,* **2009,** *5,* 741-749.

11. E. Kharlampieva, J. M. Slocik, S. Singamaneni, N. Poulsen, N. Kroger, R. R. Naik, V. V. Tsukruk, Protein-enabled Synthesis of Monodisperse Titania Nanoparticles on and within Polyelectrolyte Matrices, *Adv. Funct. Mater.*, **2009,** *19,* 2303-2311.

12. E. Kharlampieva, D. Zimnitsky, M. Gupta, K. N. Bergman, D. L. Kaplan, R. R. Naik, V. V. Tsukruk, Redox-active ultrathin template of silk fibroin: effect of secondary structure on gold nanoparticle reduction, *Chem. Mater.* **2009,** *21,* 2696-2704.

13. E. Kharlampieva, V. Kozlovskaya, J. Chan, J. F. Ankner, V. V. Tsukruk,, Spin-Assisted Layer-by-Layer Assembly: Variation of Stratification as Studied with Neutron Reflectivity, *Langmuir,* **2009,** 25, 14017

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19. K. D. Anderson, J. M. Slocik, M. E. McConney, J. O. Enlow, R. Jakubiak, T. J. Bunning, R. R. Naik, V. V. Tsukruk, Facile Plasma Enhanced Deposition of Ultrathin Crosslinked Amino Acid Films for Conformal Biometallization, *Small,* **2009,** *5,* 741-749.

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24. E. Kharlampieva, J. M. Slocik, T. Tsukruk, R. R. Naik, V. V. Tsukruk, Polyaminoacid-induced growth of metal nanoparticles on layer-by-layer templates, *Chem. Mater.*, **2008**, *20*, 5822-5831.

25. B. Weintraub, S. Chang, S. Singamaneni, W. H. Han, Y. J. Choi, J. Bae, M. Kirkham, V. V. Tsukruk, Y. Deng, Density-Controlled, Solution-Based Growth of ZnO Nanorod Arrays via Layer-by-Layer Polymer Thin Films for Enhanced Field Emission, *Nanotechnology,* **2008,** *19*, 435302.

26. S. Xu, Y. Wei, M. Kirkham, J. Liu, W. Mai, D. Davidovic, R. L Snyder, Z. L. Wang, Patterned Growth of Vertically Aligned ZnO Nanowire Arrays on Inorganic Substrates at Low Temperature without Catalyst *J. Am. Chem. Soc.*, **2008**, *130*, 14958-14959.

30+ presentations have been delivered in USA, Europe, and Asia by the PI and his students at professional conferences and seminars.

Honors, fellowships, and professional services relevant to the project
Students:

- NRC post-doctoral Fellowship (McConney, 2009)
- ACS Best Paper Award for Undergraduate Research in Polymers (Chan, 2010)
- SAIC Best Paper Award (Gupta, 2009)
- MSE Research Initiation Award (Gupta, 2010)
- NDSEG Graduate Fellowship (Combs, 2010).

PI:
- Fellow, Materials Research Society, 2011
- Humboldt Research Award, 2009
- Fellow, American Physical Society, 2010
- Member, Editorial Board, *Langmuir,* 2010
- Member, Editorial Board, *ACS Applied Materials & Interfaces,* 2010